Published in final edited form as:
Alcohol Clin Exp Res. 2008 June ; 32(6): 1023–1030.

The Tachykinin Receptor 3 Is Associated With Alcohol and Cocaine Dependence

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Abstract

Background—A broad region on chromosome 4q was previously linked to the phenotype of alcohol dependence in the Collaborative Study on the Genetics of Alcoholism sample. A strong positional candidate gene was identified within this region: tachykinin receptor 3 gene (*TACR3*), which encodes tachykinin receptor 3 (NK3R), the receptor for the tachykinin 3 (neurokinin B) peptide. Pharmacological studies have provided evidence that the administration of NK3R agonists attenuates the intake of alcohol and NK3R can also mediate the acute and chronic behavioral effects of cocaine.

Methods—Thirty SNPs were genotyped throughout *TACR3*. Family based association analysis was performed in 219 European American families to detect an association with alcohol dependence. Subsequent analyses were performed to evaluate the evidence of association with other definitions of alcohol dependence as well as cocaine dependence.

Results—Seven of the 9 SNPs in the 3′ region of *TACR3* provided significant evidence of association with alcohol dependence ($p \leq 0.05$). Further analyses suggest that the evidence of association is strongest among those subjects with more severe alcohol dependence (defined by ICD-10) and those with co-morbid cocaine dependence. Haplotype analyses further strengthen the evidence of association in the 3′ region of the gene.

Conclusions—These results indicate that sequence variations in *TACR3* contribute to the variation in more severe alcohol dependent individuals and those who are also cocaine dependent.

Keywords

Alcoholism; Association; Tachykinin Receptor 3 Gene; SNP; Family Study

Alcohol dependence is a common and debilitating disorder with a 12 month prevalence of 3.8%, which represents 7.9 million Americans (Grant et al., 2004). Genetic factors have been shown to play an important role in alcohol dependence (Cadoret et al., 1980; Cloninger et al., 1981; Goodwin, 1979; Heath et al., 1997; Kendler et al., 1994; Pickens et al., 1991). Several genes, including *GABRA2*, (Covault et al., 2004; Edenberg et al., 2004; Fehr et al., 2006; Lappalainen et al., 2005), *ADH4* (Edenberg et al., 2006; Guindalini et al., 2005; Luo et al., 2005b) and *CHRM2* (Luo et al., 2005a; Wang et al., 2004) have now been shown in multiple studies to be associated with alcohol dependence. Additional genes that are yet to be identified or replicated must also contribute to the risk of disease.

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The Collaborative Study on the Genetics of Alcoholism (COGA) has collected multiplex, alcohol dependent families in order to maximize the ability to detect genes contributing to the risk of alcoholism. Genome wide analysis demonstrated linkage to chromosome 4q (Reich et al., 1998; Williams et al., 1999). The linked region was relatively large, encompassing about 50 cm, and therefore it was hypothesized that multiple genes within this chromosomal region might contribute to the risk of alcohol dependence. We have already reported that *ADH4*, which is located within this linked chromosomal region, is associated with alcoholism in the COGA sample (Edenberg et al., 2006). Another gene, *SNCA*, was found to be associated with the craving for alcohol but was not associated with alcohol dependence itself (Foroud et al., 2007). Association studies of additional candidate genes within this region of chromosome 4q have continued.

A potential positional candidate gene within the linked region on chromosome 4q25 is *TACR3*, which encodes the tachykinin receptor 3, receptor for the tachykinin 3 (neurokinin B) peptide. *TACR3* belongs to a family of genes that encodes proteins that function as receptors for tachykinins (reviewed in Page, 2005). There are 5 known mammalian tachykinins: substance P, neurokinin A, neurokinin B, neuropeptide K and neuropeptide γ. All have a common C-terminal sequence Phe-X-Gly-Leu-Met-NH2; their affinities for receptors are based on differences at the amino terminal end. There are 3 distinct G protein-coupled receptors which bind to the tachykinins: NK1R, NK2R, and NK3R; their endogenous ligands are substance P, neurokinin A, and neurokinin B, respectively. NK1R and NK3R are both widely distributed in the brain. Neurokinin B preferentially binds to the tachykinin 3 receptor (NK3R).

Pharmacological studies have provided evidence that the administration of NK3R receptor agonists attenuates the intake of alcohol and can also mediate the acute and chronic behavioral effects of cocaine. Studies in the Sardinian preferring and nonpreferring rats found that administration of senktide, a selective NK3R agonist, inhibited alcohol intake, without affecting overall water or food intake (Ciccocioppo et al., 1994, 1995). It was hypothesized from these studies as well as others that the NK3R agonist acts to reduce ethanol consumption by replacing the rewarding properties of alcohol (Massi et al., 2000). Studies in Wistar rats have also shown that administration of an NK3R agonist blocked the acute locomotor stimulant effects of cocaine (Jocham et al., 2006). In these animals, the agonist also potentiated the cocaine-induced increase in dopamine levels in the nucleus accumbens core but did not have this effect in the nucleus accumbens shell (Jocham et al., 2006). However, species differences have been found between the rat and mouse NK3R and the human NK3R. Studies in marmoset monkeys have provided evidence that administration of an NK3R agonist, senktide, enhanced the effects of cocaine on locomotor activity, but reduced other cocaine-influenced behaviors such as exploratory activity and terrestrial glance behavior (De Souza Silva et al., 2006b). In contrast, pretreatment with a NK3R antagonist, SR142801, enhanced the aversive behavioral effects of cocaine in the monkeys (De Souza Silva et al., 2006a).

Based on both the linkage evidence as well as the studies in animal models, we hypothesized that variation in *TACR3* is associated with the susceptibility for alcohol dependence. To test this hypothesis, we genotyped SNPs throughout *TACR3* and performed family based association analysis. After finding significant evidence of association using DSM-IV diagnostic criteria, we further characterized the evidence of association by examining the effects of alcohol dependence severity and cocaine dependence on this association.

METHODS

Sample

Multiplex alcohol dependent families were recruited by COGA, an ongoing multi-site study that has recruited families at 6 centers across the United States: Indiana University, State

University of New York Downstate Medical Center, University of Connecticut, University of Iowa, University of California/San Diego, and Washington University, St. Louis. This study was approved by the institutional review boards of all participating institutions.

Alcohol dependent probands were identified through alcohol treatment programs. A poly-diagnostic interview instrument, the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) (Bucholz et al., 1994; Hesselbrock et al., 1999), was administered to the proband and their family members (see Foroud et al., 2000, and Reich et al., 1998; for more details). A subset of families having at least 3 first degree relatives who met both lifetime DSM-IIIR criteria for alcohol dependence (American Psychiatric Association and Work Group to Revise DSM-III, 1987) and lifetime Feighner (Feighner et al., 1972) criteria for definite alcoholism, participated in the genetic phase of this study. Due to the known racial differences in SNP allele frequencies and patterns of linkage disequilibrium, the analytic sample was restricted to the 219 European American families (n = 1,923 genotyped individuals).

Phenotypes

Data from the SSAGA interview were used to classify subjects as alcohol dependent (Table 1); because the linkage in this chromosome 4 region was strongest for the diagnosis based on DSM-IV criteria (American Psychiatric Association and Task Force on DSM-IV, 2000), that was our primary diagnosis. A total of 753 subjects were defined as alcohol dependent and all others with SSAGA data were classified as unaffected (n = 1,047). Following initial evidence of association using the primary phenotype (DSM-IV alcohol dependence), secondary analyses were also performed using a narrower and broader definition of alcohol dependence. For the narrower disease phenotype, those participants meeting criteria for ICD-10 were considered as affected (n = 565; Table 1), and all others with SSAGA data were classified as unaffected (n = 1,235). For the broader phenotype, subjects meeting both DSM-IIIR criteria for alcohol dependence (American Psychiatric Association and Work Group to Revise DSM-III, 1987) and Feighner (Feighner et al., 1972) criteria for definite alcoholism were classified as meeting the COGA alcohol dependence phenotype (n = 884; Table 1; all others with a SSAGA were classified as unaffected (n = 916). The 123 individuals who provided a blood sample for DNA but did not have a completed SSAGA were classified as unknown for all models of alcohol dependence.

Given the evidence from animal studies described above, we hypothesized that variations in *TACR3* could also affect cocaine dependence. However, having already identified an association with alcohol dependence, this required careful analysis to avoid confounding. Within the genotyped study sample were 255 subjects who met DSM-IIIR criteria for cocaine dependence (Table 1). Among these, 208 also met criteria for DSM-IV alcohol dependence (82%). However, 545 of the 753 DSM-IV alcohol dependent subjects (72.4%) did not meet criteria for DSM-IIIR cocaine dependence. Therefore, to explore whether the evidence of association of *TACR3* with alcohol dependence was due to the inclusion of subjects with cocaine dependence, we performed 2 additional analyses. First, we repeated the family based association analysis classifying as affected only those subjects who met criteria for cocaine dependence without regard for alcoholism status (n = 255). All others with SSAGA data were classified as unaffected, including individuals with DSM-IV alcohol dependence who were not DSM-IIIR cocaine dependent. Second, we performed family based association analysis including as affected only those individuals who met criteria for DSM-IV alcohol dependence but who did *not* meet DSM-IIIR criteria for cocaine dependence. In this way, we could determine whether the observed association of *TACR3* with alcohol dependence was due to the inclusion of subjects who were comorbid for cocaine dependence.

SNP Selection and Genotyping

Tachykinin receptor 3 gene spans 130 kb on chromosome 4q25 and contains 5 exons (Fig. 1*A*). SNPs distributed throughout the gene were selected from public databases, primarily dbSNP (http://www.ncbi.nlm.nih.gov/SNP/). Most SNPs were located in noncoding regions of the gene. Two known coding SNPs, rs2276973 in exon 3 and rs17033889 in exon 5, were also genotyped. The location of each SNP was determined from the annotations in the NCBI human genome assembly Build 36.1. At the time some SNPs were selected, allele frequencies were not available. To determine allele frequencies and to test the quality of the assays, SNPs were genotyped in 2 sets of samples, each consisting of 40 unrelated individuals from the Coriell European- and African-American diversity samples; only SNPs in Hardy Weinberg equilibrium in both test groups were genotyped on the COGA sample.

Genotyping of the 30 SNPs was done using a modified single nucleotide extension reaction with allele detection by mass spectrometry (Sequenom MassArray system; Sequenom, San Diego, CA). The assays were designed and run with either of 2 formats, hME™ or iPLEX™ (Sequenom, San Diego, CA). All SNP genotypes were checked for Mendelian inheritance using the program PEDCHECK (O'Connell and Weeks, 1998). Marker allele frequencies and heterozygosities were computed in the COGA sample using the program USERM13, part of the MENDEL linkage computer programs (Boehnke, 1991). No marker deviated significantly ($p < 0.01$) from Hardy Weinberg equilibrium.

Statistical Analyses

SNP coverage across *TACR3* was evaluated using the program HAPLOVIEW (Barrett et al., 2005), which examined the extent of linkage disequilibrium (LD) between pairs of SNPs. The program Tagger (de Bakker et al., 2005) (http://www.broad.mit.edu/mpg/tagger) was used to estimate how well the SNPs genotyped in this study represented the genetic information contained in nongenotyped SNPs. Because 7 of the SNPs genotyped in this study were not in the HapMap database and therefore could not be evaluated by Tagger, this method underestimates the extent to which the genotyped SNPs in this study also carry information on the nongenotyped variation in the gene.

The Pedigree Disequilibrium Test (PDT) (Martin et al., 2000), as implemented in the program UNPHASED (Dudbridge, 2003), was used to test for association with alcohol dependence in the extended, multiplex COGA pedigrees. The PDT utilizes data from all available trios in a family, as well as from discordant sibships. Evidence for association is assessed based on: 1) the overtransmission of a particular allele to affected individuals; and 2) the greater frequency of the allele in affected individuals as compared to their unaffected siblings. Results from the "avg-PDT" statistic, which weighs each family equally in computing the overall test statistic (Martin et al., 2001), are reported. Due to the extremely limited power to detect association, 2 SNPs with very low MAF (rs17033889 and rs2276973) were not included in the family based association analyses.

In regions of *TACR3* where association analyses with individual SNPs were significant, multi-SNP haplotypes were constructed to further analyze the evidence of association. Haplotypes were estimated using consecutive, overlapping sets of 3 adjacent SNPs (Edenberg et al., 2004). Due to the small sample size of the cocaine phenotype, missing genotypes were imputed employing the EM algorithm as per the missing option in UNPHASED. In each set of sliding windows, haplotypes having a frequency of at least 5% were used to test for association using the PDT and the global p-value is reported for each test of association. The haplotypes were then reviewed in each of the sequential sliding windows to determine whether significant association results were due to the overtransmission of a particular haplotype to affected individuals or to the differential transmission to siblings discordant for the phenotype.

RESULTS

Thirty SNPs were genotyped across the 130 kb region containing *TACR3*, extending more than 10 kb beyond the gene on both the 5′ and 3′ ends (Fig. 1*A*). Statistical analyses were not performed for the 2 exonic SNPs with very low minor allele frequency (rs17033889 and rs2276973; Table 2). Evaluation of the SNP positions (Fig. 1*A*) and the pairwise LD in our sample (Fig. 1*B*) indicated that the 28 SNPs we included in the statistical analyses adequately covered *TACR3* and its flanking regions (mean max $r^2 = 0.87$). To further evaluate the coverage of *TACR3*, we used the program Tagger (de Bakker et al., 2005) to calculate LD between the SNPs we genotyped and all 83 SNPs in the region that were analyzed by HapMap. Of the 28 SNPs analyzed, 25 were in the HapMap database and therefore could be evaluated. The average r^2 of these 25 SNPs with all 83 known HapMap SNPs (MAF ≥ 0.1) in the region was 0.87; r^2 was greater than 0.5 for 83% of the SNPs and greater than 0.8 for 80% of the SNPs. Given that we genotyped an additional 3 SNPs not in the HapMap database (and thus not incorporated into this calculation), our actual coverage was even better.

Table 2 presents the results from family based tests of association (PDT) between each *TACR3* SNP and alcohol dependence (DSM-IV criteria). Seven of the 9 SNPs in the 3′ region of *TACR3* provided significant evidence of association ($p \leq 0.05$). One marker, rs2868397, had a minor allele frequency of only 4% and therefore provided minimal power to detect association. Another SNP, rs1813782, which was informative, did not provide evidence of association ($p = 0.81$), although the adjacent SNPs both did ($p = 0.02$). Review of the LD pattern indicates that this SNP is in lower LD with its flanking markers, which were in high LD with each other. The other SNPs distributed throughout the 5′ and coding regions of *TACR3* did not provide significant evidence of association (all $p > 0.12$).

To further explore the phenotypes that contribute to the observed association, analyses were performed using narrower (ICD-10) and broader (COGA; DSM-IIIR plus Feighner definite) definitions of alcohol dependence. The same SNPs in the 3′ region of the *TACR3* gene which were significant when analyzing the DSM-IV alcohol dependence phenotype were also significant when analyses were performed using the narrower ICD-10 criteria, suggesting that the association is driven by the subset of alcoholics with a more severe phenotype. In contrast, only 1 SNP, rs4274850, was significant ($p = 0.05$) when analyses were performed using the broader alcohol dependence definition (COGA).

Because there are data from animal models suggesting that TACR3 might play a role in cocaine dependence, we carried out subsequent analyses to determine whether the association of *TACR3* with alcohol dependence was due to the inclusion of 208 individuals who were also cocaine dependent. To examine the role of cocaine dependence on the association with alcohol dependence, the family based association analysis was repeated; however, those who met criteria for both DSM-IV alcohol dependence and cocaine dependence were excluded from the sample of affected individuals ($n = 208$) to see if there was association with alcoholism in the absence of confounding by cocaine dependence. Therefore, affected individuals included only those who met DSM-IV criteria for alcohol dependence but did not meet criteria for DSM-IIIR cocaine dependence. In this analysis, 2 SNPs were significant ($p \leq 0.05$) (Table 2).

Association analysis employing cocaine dependence as the phenotype of interest detected evidence of association throughout an extended region of *TACR3*, including the 3′ region identified when analyzing the alcohol dependence phenotype and also SNPs in introns 1 and 3 (Table 2). Haplotypes were then constructed throughout *TACR3* using overlapping sets of 3 adjacent SNPs. Significant evidence ($p \leq 0.05$) of association was observed with multiple sets of consecutive haplotypes when performing analyses with DSM-IIIR cocaine dependence (Table 3). One other 3 SNP sliding window (SNP 12-14) also provided significant evidence

of association ($p = 0.01$). Overall association results and associated haplotypes were similar when analyses were performed using DSM-IV and ICD-10 alcohol dependence (Tables 4 and 5).

DISCUSSION

Linkage analyses were initially used to identify chromosome 4q25 as a region likely to harbor gene(s) contributing to alcohol dependence. Examination of this chromosomal interval identified a positional candidate gene, *TACR3*, which had been studied in rat and monkey models and found to have a role in the response to ethanol and cocaine. Based on these lines of evidence, we initially tested for the association of SNPs within the *TACR3* gene and alcohol dependence, defined by DSM-IV criteria (for which the linkage signal was strongest). Having found positive evidence of association, we then pursued secondary analyses to better delineate whether the evidence of association was found throughout the COGA sample or could be limited to a particular subset of the COGA sample. These analyses suggested that variation in *TACR3* is associated with more severe alcoholism, as demonstrated by the stronger association when analyzing ICD-10 alcohol dependence, despite the smaller number of affected individuals. The strongest evidence of association was found with the phenotype of cocaine dependence. However, 82% of the cocaine-dependent individuals were also alcohol dependent; because there were only 47 subjects who were cocaine dependence but not DSM-IV alcohol dependent, we did not have sufficient power to test whether the association with cocaine dependence was independent of alcohol dependence. In previous work (Dick et al., 2007), we found that the individuals in our study with comorbid alcohol and illicit drug dependence had more severe alcohol problems; therefore, this finding reinforces the idea that variants in *TACR3* are associated with more severe forms of alcoholism.

There is substantial clinical heterogeneity in the phenotypic expression of alcohol dependence, and it is not surprising that particular genes may contribute to subsets of the disease, defined by severity or other comorbid addictions. In previous analyses, we have detected association to *SNCA* which was observed only when using the phenotype of alcohol craving rather than alcohol dependence (Foroud et al., 2007). Comorbid addiction to drugs other than alcohol is observed among a substantial proportion of alcohol dependent individuals; within the broader COGA sample, including individuals with a less strong family history of alcohol dependence who were not included in the genotyped sample reported herein, there were 1,964 subjects meeting DSM-IV criteria for alcohol dependence. Within this group, 32% also met criteria for DSM-IIIR cocaine dependence.

The primary phenotype analyzed for association with *TACR3* was DSM-IV alcohol dependence. Three additional phenotypes were also analyzed as part of secondary analyses (COGA, ICD-10 and cocaine dependence). Thus, we have 3 nested alcohol dependence phenotypes and 1 cocaine dependence phenotype; the latter includes many subjects who are also alcohol dependent. It is difficult to assess the number of independent tests; however, we propose that there are at most 2 independent tests being performed. One is a test of the association with alcohol dependence, and the other is a test of the association with cocaine dependence. Employing a conservative Bonferroni adjustment for 2 phenotypic groups, our adjusted significance threshold would be $0.05/2 = 0.025$. Using this criterion, 3 SNPs were significantly associated with DSM-IV alcohol dependence and 4 SNPs were significantly associated with ICD-10 alcohol dependence. Nine SNPs were significantly associated with cocaine dependence. Furthermore, 5 sliding windows [1–3, 2–4, 3–5, 4–6, and 5–7] were significantly associated with ICD-10 alcohol dependence and 2 windows were significantly associated with cocaine dependence [4–6 and 5–7].

This study has several strengths. First, 30 SNPs were genotyped throughout *TACR3*, allowing rigorous evaluation of the evidence of association. Second, a large sample of 219 European American multiplex alcohol dependent families were analyzed using family based methods of association analysis, reducing the potential errors due to stratification. Third, the depth of phenotyping completed in the vast majority of the genotyped individuals allowed us to determine that the association was due to the most severely affected alcoholics who were also cocaine dependent.

This study did also have limitations. We did not have a large sample of African American families and therefore are not able to confirm whether this association would also replicate in ethnically diverse samples. We also did not have a large sample of individuals who were only cocaine dependent; therefore, we cannot test whether variation in *TACR3* can affect the risk of cocaine dependence independently of the association with alcohol dependence.

In summary, we have detected consistent evidence of association in the COGA sample in the 3′ region of *TACR3* when analyzing alcohol dependence as well as cocaine dependence. Our results suggest that the evidence of association is primarily due to that subset of the sample that meets more severe criteria for alcohol dependence (DSM-IV and ICD-10) and are both alcohol dependent and cocaine dependent. These results confirm the data in the rat and monkey which suggested that the NK3 receptor plays an important role in the response to ethanol and cocaine. Studies in additional samples are critical to confirm these results and better delineate how variation in *TACR3* mediates its effects.

Acknowledgements

The Collaborative Study on the Genetics of Alcoholism (COGA), Co-Principal Investigators B. Porjesz, V. Hesselbrock, H. Edenberg, L. Bierut, includes 9 different centers where data collection, analysis, and storage take place. The 9 sites and Principal Investigators and Co-Investigators are: University of Connecticut (V. Hesselbrock); Indiana University (H.J. Edenberg, J. Nurnberger Jr., P.M. Conneally, T. Foroud); University of Iowa (S. Kuperman, R. Crowe); SUNY Downstate (B. Porjesz); Washington University in St. Louis (L. Bierut, A. Goate, J. Rice); University of California at San Diego (M. Schuckit); Howard University (R. Taylor); Rutgers University (J. Tischfield); Southwest Foundation (L. Almasy). Zhaoxia Ren serves as the NIAAA Staff Collaborator. This national collaborative study is supported by the NIH Grant U10AA008401 from the National Institute on Alcohol Abuse and Alcoholism (NIAAA) and the National Institute on Drug Abuse (NIDA).

Genotyping facilities were provided by the Center forMedical Genomics at Indiana University School of Medicine, supported in part by the Indiana Genomics Initiative (INGEN, supported in part by the Lilly Endowment, Inc.). We thank Gayathri Rajan and Rachel Thowe for their superb technical support on SNP genotyping.

In memory of Henri Begleiter and Theodore Reich, Principal and Co-Principal Investigators of COGA since its inception; we are indebted to their leadership in the establishment and nurturing of COGA, and acknowledge with great admiration their seminal scientific contributions to the field.

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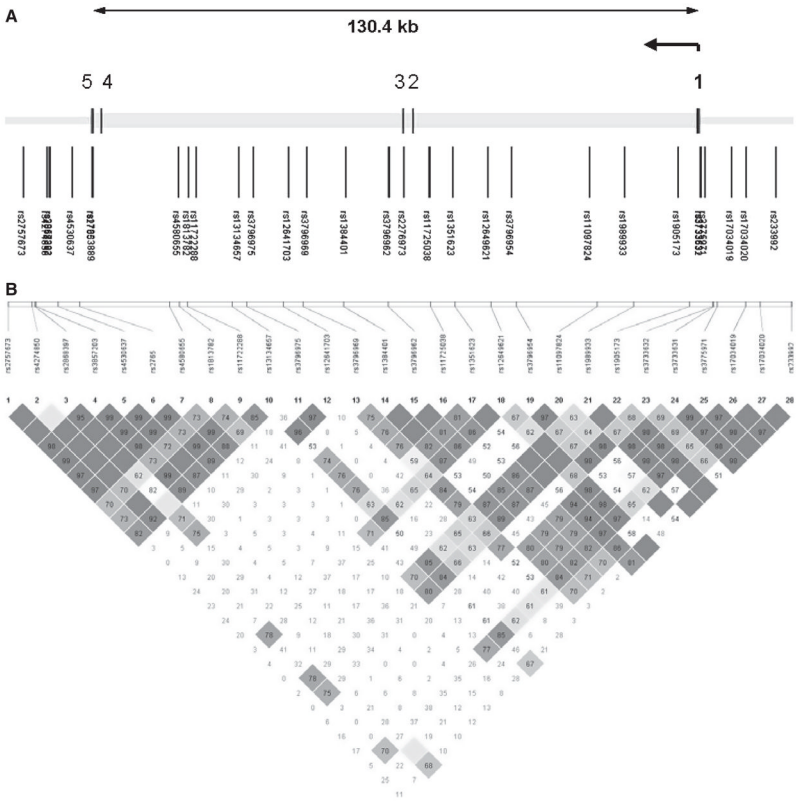

Fig. 1.

(A) Genomic structures of *TACR3*. The gene structure of *TACR3* is based on transcript NM_001059. The direction of transcription and the exons are indicated by arrows and numerical numbers, respectively. The size of the gene is indicated in scale at the top of the figure. **(B)**. Pairwise linkage disequilibrium. Pairwise linkage disequilibrium (D′) estimates among the genotyped SNPs. Darkly shaded boxes have strong evidence of linkage disequilibrium, defined as a pair of SNPs with the one-sided upper 95% confidence bound on D′ of 0.98 and the lower bound above 0.7. Lightly shaded boxes have lower linkage disequilibrium.

Table 1

Phenotypic Characteristics of the Genotyped Individuals[a]

Phenotypes	Male	Female	Total
Alcohol dependence			
DSM-IV alcohol dependence	499	254	753
ICD-10 alcohol dependence (Narrow)	389	176	565
DSM-IIIR alcohol dependence + Feighner definite alcohol dependence (Broad)	590	294	884
Cocaine dependence			
DSM-IIIR cocaine dependent	161	94	255
Comorbid			
DSM-IV alcohol dependent + DSM-IIIR cocaine dependent	142	66	208
ICD-10 alcohol dependent + DSM-IIIR cocaine dependent	126	57	183

[a]There are 123 individuals, 64 males and 59 females, who have genotypic information but who did not complete a SSAGA and therefore are classified as unknown for all phenotypes being analyzed.

Table 2

Association of *TACR3* SNPs With Study Phenotypes

SNP ID	Position[a]	SNP location[b]	MAF[c]	HWE[d]	Alcohol dependence			DSM-IV Alc Dep no cocaine[e]	DSM-IIIR cocaine,[ef]
					DSM-IV[e]	ICD-10[e]	COGA[e]		
[1] rs2757673	104715499	Downstream	0.25	1.00	0.02	0.02	0.11	0.10	0.02
[2] rs4274850	104720470	Downstream	0.19	0.87	0.05	0.01	0.05	0.05	0.11
[3] rs2868397	104720987	Downstream	0.04	0.79	0.26	0.23	0.25	0.61	0.03
[4] rs3857203	104721232	Downstream	0.35	0.55	0.04	0.04	0.16	0.11	0.01
[5] rs4530637	104725838	Downstream	0.34	1.00	0.03	0.02	0.15	0.11	0.01
[6] rs2765	104730215	3′UTR	0.33	0.66	0.03	0.02	0.15	0.19	0.007
rs17033889	104730341	Exon 5, Thr449Ala	0.01	1.00	N/A[g]	N/A	N/A	N/A	N/A
[7] rs4580655	104748723	Intron 3	0.34	0.44	0.02	0.03	0.11	0.12	0.009
[8] rs1813782	104750764	Intron 3	0.37	0.79	0.81	0.46	0.95	0.63	0.04
[9] rs11722288	104752399	Intron 3	0.29	0.94	0.02	0.03	0.12	0.04	0.02
[10] rs13134657	104761590	Intron 3	0.11	0.64	0.31	0.70	0.93	0.56	0.35
[11] rs3796975	104764604	Intron 3	0.45	0.44	0.75	0.94	0.85	0.55	0.63
[12] rs12641703	104772136	Intron 3	0.49	0.87	0.20	0.46	0.66	0.19	0.51
[13] rs3796969	104776115	Intron 3	0.37	0.76	0.16	0.60	0.45	0.07	0.12
[14] rs1384401	104784527	Intron 3	0.35	0.05	0.90	0.57	0.62	0.47	0.02
[15] rs3796962	104793714	Intron 3	0.34	0.14	0.70	0.26	0.43	0.71	0.01
rs2276973	104796831	Exon 3, Arg286Lys	0.00	1.00	N/A	N/A	N/A	N/A	N/A
[16] rs11725038	104802382	Intron 1	0.34	0.08	0.90	0.34	0.80	0.39	0.006
[17] rs1351623	104807426	Intron 1	0.17	0.24	0.18	0.92	0.92	0.21	0.12
[18] rs12649621	104814831	Intron 1	0.10	0.70	0.56	0.94	0.95	0.93	0.57
[19] rs3796954	104820021	Intron 1	0.13	0.02	0.43	0.65	0.92	0.40	0.76
[20] rs11097824	104836637	Intron 1	0.10	0.99	0.76	0.55	0.15	1.00	0.49
[21] rs1989933	104844112	Intron 1	0.10	0.68	0.53	0.92	0.91	0.89	0.57
[22] rs1905173	104855551	Intron 1	0.09	0.93	0.52	0.99	0.79	0.93	0.54
[23] rs3733632	104860384	5′UTR	0.20	0.02	0.23	0.85	0.88	0.08	0.71
[24] rs3733631	104860552	Promoter	0.16	0.73	0.52	0.84	0.74	0.63	0.42
[25] rs3775971	104861369	Promoter	0.16	0.67	0.39	0.89	0.91	0.42	0.33
[26] rs17034019	104867106	Upstream	0.10	0.69	0.66	0.83	0.84	0.92	0.46
[27] rs17034020	104870122	Upstream	0.06	1.00	0.88	0.88	0.50	0.60	0.46
[28] rs233992	104876519	Upstream	0.44	0.19	0.12	0.90	0.14	0.16	0.43

[a] Chromosome positions are based on NCBI Human Genome Assembly v. 36.1.

[b] SNP location relative to the exons of *TACR3*, based on transcript NM_001059.1. Transcription is opposite to the human genome direction.

[c] Minor allele frequency in European Americans.

[d] *p*-Value for test of Hardy Weinberg equilibrium.

[e] *p*-Value of avg-PDT statistic for associations between the SNPs and phenotypes shown.

[f] Note that 82% of individuals with cocaine dependence were also alcohol dependent.

[g] N/A not available due to very low MAF resulting in no power to detect evidence of association.

Alcohol Clin Exp Res. Author manuscript; available in PMC 2008 June 18.

Table 3

Association of *TACR3* Haplotypes With Cocaine Dependence

	SNP [1-3]		SNP [2-4]		SNP [3-5]		SNP [4-6]		SNP [5-7]		SNP [6-8]	
Overtransmission	**A**[a]	**U**[b]	**A**	**U**	**A**	**U**	**A**	**U**	**A**	**U**	**A**	**U**
[1] rs2757673	G	A										
[2] rs4274850	A	C	A	C								
[3] rs2868397	C	C	C	C	C	C						
[4] rs3857203			G	A	G	A	G	A				
[5] rs4530637					G	A	G	A	G	A		
[6] rs2765							A	G	A	G	A	G
[7] rs4580655									A	G	A	G
[8] rs1813782											A	G
[9] rs11722288												
Haplotype (*p*-value)[c]	0.09	0.09	0.07	0.08	0.05	0.04	0.02	0.007	0.02	0.01	0.17	0.03
Global (*p*-value)[d]	0.09		0.10		0.05		0.01		0.01		0.12	

[a] DSM-IIIR cocaine dependence.

[b] Unaffected.

[c] *p*-Value associated with the differential transmission of that haplotype.

[d] Global *p*-value for the PDT using all haplotypes observed with these 3 SNPs.

Table 4

Association of *TACR3* Haplotypes With Alcohol Dependence (DSM-IV)

	SNP [1-3]		SNP [2-4]		SNP [3-5]		SNP [4-6]		SNP [5-7]		SNP [6-8]	
Overtransmission	**A**[a]	**U**[b]	**A**	**U**	**A**	**U**	**A**	**U**	**A**	**U**	**A**	**U**
[1] rs2757673	G	A										
[2] rs4274850	A	C	A	C								
[3] rs2868397	C	C	C	C	C	C						
[4] rs3857203			G	A	G	A	G	A				
[5] rs4530637					G	A	G	A	G	A		
[6] rs2765							A	G	A	G	A	G
[7] rs4580655									A	G	A	G
[8] rs1813782											A	G
[9] rs11722288												
Haplotype (*p*-value)[c]	0.02	0.02	0.04	0.08	0.15	0.15	0.07	0.07	0.03	0.03	0.22	0.06
Global (*p*-value)[d]	0.02		0.05		0.15		0.07		0.03		0.18	

[a] DSM-IV alcohol dependence.

[b] Unaffected.

[c] *p*-Value associated with the differential transmission of that haplotype.

[d] Global *p*-value for the PDT using all haplotypes observed with these 3 SNPs.

Table 5

Association of *TACR3* Haplotypes With Alcohol Dependence (ICD-10)

	SNP [1-3]		SNP [2-4]		SNP [3-5]		SNP [4-6]		SNP [5-7]		SNP [6-8]	
Overtransmission	**A**[a]	**U**[b]	**A**	**U**	**A**	**U**	**A**	**U**	**A**	**U**	**A**	**U**
[1] rs2757673	G	A										
[2] rs4274850	A	C	A	C								
[3] rs2868397	C	C	C	C	C	C						
[4] rs3857203			G	A	G	A	G	A				
[5] rs4530637					G	A	G	A	G	A		
[6] rs2765							A	G	A	G	A	G
[7] rs4580655									A	G	A	G
[8] rs1813782											A	G
[9] rs11722288												
Haplotype (*p*-value)[c]	0.02	0.02	0.02	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.08	0.04
Global (*p*-value)[d]	0.02		0.01		0.02		0.01		0.007		0.09	

[a] ICD-10 alcohol dependence.

[b] Unaffected.

[c] *p*-Value associated with the differential transmission of that haplotype.

[d] Global *p*-value for the PDT using all haplotypes observed with these 3 SNPs.